auto-plasty, there has been practiced a procedure in which a prosthetic material for defected sites of cartilage and bone composed of a combination of a bone morphogenetic protein and a suitable carrier was imbedded in the defected site. In practicing this, the defected side can be exposed on surgical operation to apply a cartilage and bone repairing composition containing a bone morphogenetic protein directly to the defeced site, and thus, the materials in a solid form such as blocks, sponges, sheets and the like which are easy to handle have been widely applied. Those in a semisolid form such as gels or pastes can also be used. As the carriers which make such solid or semisolid forms applicable, there have been utilized, for example, metals such as stainless or titanium alloys or collagen and hydroxyapatite (HAP) or a mixture thereof.

K1 cont.

Please replace the paragraph bridging pages 3 and 4 with the following:

--The present inventors have made earnest studies on the relationship between the active ingredient, a bone morphogenetic protein, and a carrier therefor in the case of a bone repairing method without surgical operation and have found that a certain class of polyoxyethylene-polyoxypropylenes can show a high bio-absorption, a good affinity to a bone morphogenetic protein and temperature dependent sol-gel reversible transition. The present inventors have prepared a bone morphogenetic composition by mixign an aqueous polyoxyethylene-polyoxypropylene solution and a bone morphogenetic protein, which is an injectable liquid at a

K2

temperature of from 1°C to 30°C at the time of administration and may be gelatinized at around 37C within 3 minutes after administration. It has been found that ectopic cartilage and bone morphogenesis are accomplished by administering said compositions to mice intramuscularly at the femoral muscle and then retaining a bone morphogenetic protein at the adminstration sites in vivo, upon which this invention has been completed.--

Please replace the paragraph beginning at page 4, line 16 with the following rewritten paragraph:

--This invention is concerned with a cartilage and bone morphogenetic repairing composition which contains a polyoxyethylene-polyoxypropylene and a bone morphogenetic protein.--

Please replace the paragraph bridging pages 4 and 5 as follows:

--The polyoxyethylene-polyoxypropylene as used herein is a generic name of nonionic surface active agents of a polymer type having less hydrophilic polyoxypropylene as a hydrophobic group and ethylene oxide as a hydrophilic group. It may be feasible to prepare surface active agents having various properties by changing a molecular weight of the polyoxypropylene and a mixing ratio thereof to the ethylene oxide. The synthesizable polyoxyethylene-polyoxygropylenes have a molecualr weight of the polyoxypropylene in the range of 900-4,000 and a percent by weight of the ethylene oxide in the total molecule of 5%-90%. For

instance, the polyoxyethylene-propylene block polymers (ADEKA®) manufactured by Asahi Denka Kogyo K.K. are systematically named according to a molecular weight of polyoxypropylene and a weight ratio of the ethylene oxide to be added and the classification list thereof is shown in Fig. 1.

Please rewrite the first full paragraph of page 5 as follows:

--Industrial utilization of polyoxyethylene-polyoxypropylenes includes aperients, ointment bases, artificial blood, coating for tablets, excipients, solubilizers or solubilizing agents for injections and others in the field of pharmaceutics, in addition to the use as general cleaning agents or antifoamings. In particular, Pluronic F-68 (a molecular weight of polyoxypropylene of 1,750 and an ethylene oxide content of 80%) has a remarkable antihemolytic action and has been marketed in the name of EXOCORPOL® from the Green Cross Corporation (polyoxyethylene-polyoxypropylenes) as an additive for extracorporeal circulation of blood. It is apparent from the results of toxicity tests using various animals that polyoxyethylene-polyoxypropylenes have extremely low toxicity and low irritative property, with no reports on possible side-effects such as antigenicity and so on (Fragrance Journal, 7, 82-87, 1974). The results of toxicity tests are shown in Table 1.--

Please replace the paragraph beginning at page 6, line 29 to **page 7, line 14 with the following rewritten paragraph:**

--Polyoxyethylene-poloxypropylenes are superior in terms of handiness to collagen showing non-reversible phase-transition by changes in temperature in the point that they show reverisble sol-gel phase-transition. This property may be controlled by selection of the optimum polyoxyethylene-polyoxypropylene for the temperature to develop the phase-transition and by changing the concentration of an aqueous solution of said polyoxyethylene-polyoxypropylene (Int. J. Pharm. 22, 207-218, 1984 and EP 0551626A1).

It is obvious from the foregoing that polyoxyethylene-polyoxypropylenes have a superior nature as a drug carrier. Attempts have already been made to combine them with a low molecular weight drug such as local anesthetics, anticancer agents and son on (Int. Pharm. 8, 89-99, 1981 and Chem. Pharm. Bull. 32, 4205-4208, 1984) and to admix with a high molecular weight physiologically active protein such as interleukins and the like (Pharm. Res. 9, 425-434, 1992).

This invention relates to a cartilage and bone morphogenetic repairing composition which contains a polyoxyethylene-polyoxypropylene and a bone morphogenetic protein, wherein the polyoxypropylene as a constituent of said polyoxyethylene-polyoxypropylene has a molecular weight of about 1,500-4,000 and an ethylene oxide content of about 40-80%/molecule. Within the above ranges, there will be provided the Pluronics capable of performing temperature-dependent sol-gel reversible transition,

which characterized the present Pluronics.

Moreover, this invention relates to a cartilage and bone morphogenetic repairing composition wherein a concentration of polyoxyethylene-polyoxypropylenes as described above in an aqueous solution is about 10-50%. It is known that the reversible phase transition temperature of polyoxyethylene-polyoxypropylenes varies in general depending on the concentration of their prepared aqueous solutions, and the polyoxyethylene-polyoxypropylenes within the above-mentioned constituent ranges may gelate at around body temperature, i.e. about 37°C at a concentration of about 10-90% in its aqueous solution. As the most preferable example, there is prepared the polyoxyethylene-polyoxypropylene block polymer aqueous solution of 15-30% concentration having a molecular weight of polyoxypropylene of 3,850 and an ethylene oxide content of 70% (Pluronic F-127).

Please replace the paragraph beginning at page 7, line 19 to page 8, line 9 with the following rewritten paragraph:

--The bone morphogenetic proteins used in the this invention include, but are not limited to, a series of proteins belonging to the TGI-ß gene superfamily such as BMP-2 to BMP-9 and so on, the protein named MP52, the protein named GDF-5 and the like. Particularly preferable BMP-2 is a protein produced using Chinese hamster ovary (CHO) cells according to the genetic engineering

technology reported by Wang, et al. (Proc. Natl. Acad. Sci, USA 87, 2220-2224, 1990 and U.S. Patent No. 4,877,864), and particularly preferable MP52 is a new protein produced according to the genetic engineering technology suggested by the present inventors (our copending Japanese Patent Application Serial No. 531,621 filed October 20, 1977). This new protein can be produced by constructing a plasmid containing the DNA sequence coding the amino acid sequence as shown in SEQ ID No: 1 of the Sequence Listing derived from MP52 described in said Japanese patent application and having added the codon coding methionine at the N-terminal of said DNA sequence; transforming the plasmid into E.coli; incubating the E.coli to obtain an inclusion body; and solubilizing and purifying the inclusion body to obtain a monomer protein, which is then dimerized and purified.

KJ Cont.

An aqueous solution of 15-30% polyoxyethylene-polyoxypropylene block polymer containing as an active ingredient BMP-2 or MP52 was intramuscularly injected to mice at the femoral muscle. MP52 was retained at the adminstered sites and then an ectopic cartilage and bone morphogenesis ability was observed in vivo.

There has not yet been reported to date an injectable cartilage and bone morphogenetic repairing composition comprising a plyoxyethylene-polyoxypropylene in combination with a bone morphogenetic protein which may be useful for repair of cartilage

and bone, especially as a treating agent for bone fracture.

The present invention is further concerned with a cartilage and bone repairing composition containing a polyoxyethylene-polyoxypropylene and a bone morphogenetic protein.

K7 conclude

Moreover, the present invention is concerned with a method of treatment of a cartilage and bone repairing, by which a cartilage and bone morphogenetic composition comprising a polyoxyethylene-polyoxypropylene in combination with a bone morphogenetic protein is administered locally to the site of bone fracture or bone defect of human or animal.

Please replace the paragraph beginning at page 9, line 2 with the following:

--BRIEF DESCRIPTION OF THE DRAWINGS:

K8

Figure 1 is a classification figure for ADEKA® Pluorinics, wherein an ethylene oxide content in terms of % by weight in a toal molecule of a polyoxyethylene-polyoxypropylene is indicated on the abscissa, while a molecular weight of the compound polyoxypropylene in a polyoxyethylene-polyoxypropyleneis indicated on the ordinate.

Please rewrite theparagraph in lines 4 to 7 of page 10 as follows:

--ADEKA® Pluronic F-127 (Asahi Denka Kogyo K.K.) is known to

K9

be one of the least toxic polyoxyethylene-polyoxypropylenes ("SEIYAKU KOJO" 6, 875-880, 1986). In 7.0g of distilled water for injection was dissolved under ice-cooling 3.0g of ADEKA® Pluronic F-127 to prepare a 30% aqueous solution of ADEKA® Pluronic F-127. The aqueous solution of ADEKA® Pluronic F-127 was poured portionwise under ice-cooling to a 96-well titer plate at 360μl, 40μl of 0.01 N HCl containing 80 μg of BMP-2 was added to each well and mixed. The mixture was sterilized by passing through a 0.22μm filter to at 4°C to form a BMP-2 injection of a total volume of about 400μl (a final concentration of ADEKA® Pluronic F-127 of 27%). Similarly, the BMP-2 injections having final concentrations of ADEKA® Pluronic F-127 of 10, 15, 18 and 22.25% were prepared.

Please rewrite the paragraph of lines 8 to 13 of page 12 as follows:

--It was clearly shown in Table 2 that MP52 when polyoxyethylene-polyoxypropylene were used as a pharmaceutical carrier could apparently be retained more as compared with the case where a simple MP52 aqueous solution was injected of Example 1.

Please rewrite the paragraph of lines 16 to 18 of page 13 as follows:

--From the aforesaid results, safety and usefulness of a